

## *Mycobacterium tuberculosis* Beijing Genotype and Mycobacterial Interspersed Repetitive Unit Typing

Rao et al. (3) described a method to identify *Mycobacterium tuberculosis* strains of the Beijing genotype. The authors suggested using simple PCR of mycobacterial interspersed repetitive unit–variable-number tandem repeat (MIRU-VNTR) locus 26 to identify these strains. This suggestion is based on the authors' assumption that MIRU locus 26 has a specific seven-copy signature in these strains. Rao et al. (3) based their conclusions on 10 available Beijing strains (DNA samples) for which they indeed found seven-copy signatures. The authors justly mentioned that their test should be validated and confirmed with a large number of known Beijing strains. Prior to further experiments, it would be advisable, however, to compare their results against an already large number of available articles on MIRU typing of *M. tuberculosis* strains, published between 2001 and 2004, also containing information about Beijing strains (1–2, 4–6), including our article specially focused on the MIRU typing of the Beijing genotype (2). These articles clearly demonstrated for a large number of representative samples of strains from Russia ( $n = 44$ ), South Africa ( $n = 38$ ), Singapore ( $n = 160$ ), Bangladesh ( $n = 15$ ), and other locations that MIRU locus 26 is not monomorphic but instead moderately polymorphic in the Beijing genotype and may consist of two, three, four, five, six, seven, eight, or nine copies [1, 2, 4, 5, 6]. Furthermore, a brief check of the mentioned articles (1, 4–6) reveals that seven copies at MIRU locus 26 may occur not only in some Beijing strains but also in strains of other genotypes of *M. tuberculosis*. I am afraid that for these reasons, formally speaking, the proposed method dramatically lacks both sensitivity and specificity and cannot be used to identify *M. tuberculosis* Beijing genotype strains in any setting.

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### Authors' Reply

We accept the arguments of Igor Mokrousov, that variants of the Beijing genotype with or without seven allele copies at locus 26 have been documented, and we regret the omission of those references. The argument is based mainly on their study of Russian and South African Beijing genotypes using MIRU-VNTR typing (2). MIRU locus 26 has been promoted largely as a “Beijing-discriminating” locus for some time. However, it has been lately realized that members of the *M. tuberculosis* Beijing family are quite diverse as regards MIRU-VNTR typing results but homogeneous with respect to spoligotyping results. Mokrousov and colleagues (2) have also documented the occurrence of seven allele copies at MIRU locus 26 for all the South African isolates that they compared and a fraction of the Russian Beijing population called MIRU type 11 (M11) but not in the majority of Russian isolates. This means that locus 26 is not really a “Beijing-discriminating” locus for the majority of Russian isolates but could still be preliminarily used for a large number of native Beijing isolates circulating in other countries, such as Singapore (4).

In our study (3), we stressed that the seven allele copies at MIRU locus 26 and a spoligotype signature (hybridization corresponding to spacers 35 to 43) should together constitute a definite identification of the Beijing genotype. However, since many clinical laboratories, especially in resource-poor countries, do not have the capabilities to perform spoligotyping, we attempted to test whether a simplified MIRU locus 26-specific PCR can be used as a stand-alone test. Our findings revealed that locus 26 PCR could still be broadly applicable for most of the Beijing isolates circulating in different countries, although we now agree that it may not be a stand-alone locus for the discrimination of Beijing strains in all settings, especially the Russian setting. Given the fact that this locus has earlier been used successfully, although with a technically complicated format of allele typing on automated DNA sequencers (5), we independently attempted to simplify its use for diagnosis after validating it with a blinded collection of samples. This exercise revealed to us a good concordance of occurrence of seven alleles at locus 26 with spoligotyping when we used the genomic DNA of the strains from Libya and the Kremer collection (1). This collection is largely representative of the strains circulating throughout the world and has been tested for MIRU-VNTR typing previously (5). Indeed, the greatest shortcoming of our paper is the very small number of Beijing

samples analyzed by us. We believe, and we have mentioned explicitly in our conclusion, that a large number of Beijing strains should be tested as regards this locus alone before field level testing. We also suggested that this locus might be used in rapid diagnosis preliminarily to initiate precautionary treatments until spoligotyping data are available.

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